Cell Metabolism
Previews

# Stressed-Out Mitochondria Get MAD

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DOI 10.1016/j.cmet.2010.11.018

**Mechanisms maintaining mitochondrial integrity range from specific repair pathways to wholesale degradation of damaged organelles. A recent study in *Molecular Cell* (Heo et al., 2010) adds another mechanism to this mitochondrial homeostasis toolkit: mitochondria-associated protein degradation (MAD) that ultimately directs endoplasmic reticulum-associated protein degradation (ERAD) pathway components to oxidatively stressed mitochondria.**

Mitochondria are multifarious cellular organelles whose disruption can cause cellular dysfunction, human diseases, and age-related decline (Shadel, 2008; Wallace, 2005). Mitochondria house the ATP-producing oxidative phosphorylation (OXPHOS) system, which also generates reactive oxygen species (ROS) as products of the associated high-energy electron transfer reactions. ROS are agents of oxidative stress, and their enhanced production/accumulation promotes mitochondrial damage that can have pathogenic consequences. Thus, mechanisms to remove damaged mitochondrial components, some of which are encoded by resident mtDNA, are vital to prevent mitochondrial dysfunction, including changes in mitochondrial morphology/dynamics, intrinsic proteolytic pathways, and autophagy/mitophagy (Tatsuta and Langer, 2008) (Table 1).

Though ubiquitin was not present in the bacterial progenitors of mitochondria, this posttranslational modification is increasingly implicated in mitochondrial events (Livnat-Levanon and Glickman, 2010). However, whether a mitochondria-associated protein degradation (MAD) pathway exists that is similar to the ubiquitin-mediated endoplasmic reticulum-associated protein degradation (ERAD) pathway has not been clearly established. In a compelling new study, Heo et al. (2010) provide evidence from multiple model systems and approaches that a conserved mitochondrial protein, Vms1, interacts with and recruits the ERAD components Cdc48 and Npl4 to mitochondria in order to maintain organelle function. Inability to mount this mitochondrial stress response leads to mitochondrial respiratory failure and decreased chronological life span in yeast, highlighting the importance of the new MAD pathway.

Heo et al. (2010) show that yeast Vms1 is primarily cytosolic during growth, but localizes to the mitochondrial outer membrane in stationary phase or under oxidative stress conditions. Under these latter conditions, yeast strains lacking Vms1 show profound growth defects and decreased life span due to mitochondrial breakdown. Similarly, RNAi of the *C. elegans* Vms1 paralog does not cause growth or developmental defects, but the animals are sensitive to hydrogen peroxide and have reduced life span. Furthermore, peroxide treatment promotes Vms1p mitochondrial localization. Heo et al. (2010) purified yeast Vms1 and found it binds with Cdc48 and cofactor Npl4. Importantly, mitochondrial stress promotes the shuttling of these ERAD components to mitochondria in a Vms1-dependent manner.

Interestingly, Heo et al. (2010) also document that *VMS1* null strains are hypersensitive to the TOR kinase inhibitor rapamycin, which also causes Vms1p translocation to mitochondria. At first glance, this seems to implicate the conserved TOR signaling pathway in MAD regulation, but the authors instead favor the idea that this was a mitochondrial oxidative stress response to the drug. This conclusion was bolstered by the results of a genetic screen for suppressors of *vms1* rapamycin hypersensitivity, which uncovered three genes involved in oxidative stress resistance. This conclusion is apparently at odds with other reports showing that reduced TOR signaling extends yeast chronological life span (CLS) and reduces cellular ROS by increasing mitochondrial respiration (Bonawitz et al., 2007; Pan and Shadel, 2009). It is likely that the timing and dose of rapamycin treatment are important determinants of how this drug affects CLS, which may be the source of discrepancy between these studies. With this in mind, it is important to highlight that TORC1 signaling inhibits stress-response pathways, including those tied to oxidative stress and autophagy (De Virgilio and Loewith, 2006). Thus, TORC1 could prevent MAD from taking place as a normal part of this regulatory scheme in addition to simply responding to rapamycin-induced oxidative stress as proposed. If so, an important future goal is to determine if the effect of rapamycin on Vms1 is conserved in mammals and contributes to the life span-extending effects of reduced mTOR signaling.

To probe the in vivo relevance of the newly uncovered MAD pathway, Heo et al. (2010) examined a known substrate for ubiquitination and proteasomal decay, the mitofusin Fzo1 (Cohen et al., 2008). Absence of Vms1 reduces the rate of Fzo1 degradation and leads to its accumulation in stationary phase, suggesting that the Vms1-Cdc48-Npl4 complex extracts Fzo1 from the outer mitochondrial membrane for degradation. A unique subset of ubiquitin-conjugated proteins accumulates in *vms1* null mutant strains, pointing to distinct mitochondrial substrates for MAD. Obviously, it will be of great interest to determine the MAD substrate profile and which types and sites of ubiquitination are utilized. Finally, whether inner membrane proteins, which

**Table 1. Mitochondrial Quality Control Pathways**

| Pathway | Purpose | Examples of Specific Pathways or Proteins Involved |
|---|---|---|
| DNA repair | Maintain mtDNA stability | Base excision repair |
| Mitochondrial chaperones | Facilitate correct import/folding and prevent protein aggregation | Mitochondrial heat shock family members (e.g., HSP60) |
| Autophagy (macroautophagy) | Turnover cytoplasmic components, including mitochondria | Atg proteins (e.g., Beclin-1, LC3) |
| Mitophagy | Specific degradation of damaged mitochondria | Atg32 |
| Antioxidant defenses | Detoxify mitochondrial ROS | Mitochondrial superoxide dismutases |
| Mitochondrial fusion/fission | Interorganelle mixing/segregation of damaged mitochondria | Opa1/Mfn1,2/Drp1 |
| Mitochondrial proteolysis | Turnover of intraorganellar proteins | Lon and AAA-proteases |
| Mitochondrial-associated protein degradation (MAD) | Ubiquitin-mediated turnover of specific mitochondrial proteins | Vms1p-Cdc48p-Npl4p complex |

in principle stand a greater chance of being damaged oxidatively by mitochondrial ROS, are also substrates for this pathway should be seriously entertained. Reports that mitochondrial inner-membrane proteins are targets for degradation by the cytosolic proteasome are intriguing in this regard (Azzu and Brand, 2010).

Heo et al. (2010) provide a convincing case for a MAD mechanism (similar to ERAD) for specific degradation of oxidatively damaged mitochondrial proteins, adding to the list of important mitochondrial quality control (QC) mechanisms (Table 1). The interdependencies of repair systems, fission/fusion dynamics, autophagy/mitophagy, and specific protein degradation pathways as well as their redundancy under various stress conditions will be important to determine. For example, defects in the ubiquitin-proteasome system (UPS) lead to changes in mitochondrial dynamics (Livnat-Levanon and Glickman, 2010), possibly as a way to segregate damaged mitochondrial components for removal, and Heo et al. (2010) show that yeast lacking Vms1 are more dependent on their intrinsic mitochondrial proteases, Yme1 and Oma1, and upregulate mitophagy. Once bona fide substrates of this system are identified, answers to obvious outstanding questions can be sought. Are the targets of MAD nascent misfolded proteins (like during ERAD), oxidatively damaged mature proteins, or both? How are potential substrates inside the organelle recognized and then transported out? Which additional QC proteins recruit Vms1 to mitochondria (or vice versa) during times of stress? Are different stresses read out differentially? Are ROS or other retrograde signals involved?

Many components of the yeast and human mitochondrial proteome are conserved, and recent estimates suggest that ~20% are involved in inherited human diseases (Prokisch et al., 2006). Given the role of mitochondrial dysfunction and aberrant protein clearing in neurodegenerative diseases (Tatsuta and Langer, 2008), components of the MAD pathway are attractive candidate disease genes. Clearly, the budding yeast is still flexing its mitochondrial muscles as a model system of human biology, disease, and aging.

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